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Attention: Examiner Irene Marx

Group Art Unit 1651

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Date:

*9-17-03*

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Inventors: Rehberger et al.

Art Unit: 1651

Serial No. 09/912,049

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**TRANSMITTAL LETTER FOR PROPOSED CLAIM AMENDMENTS**

Commissioner for Patents  
P.O. Box 1450  
Arlington, VA 22313

Dear Sir:

In preparation for the telephone interview with Examiner Marx to be conducted on September 18, 2003, at 11:00 EDT, enclosed are proposed claim amendments.

The total length of this transmission, including this transmittal letter, is 16 pages. Should this transmission be incomplete or any pages thereof unintelligible, the Examiner is requested to contact the undersigned at the telephone number appearing below.

Respectively submitted,

*Mary E. Eberle*

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Date: September 17, 2003

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**PROPOSED AMENDMENTS**

1. (Withdrawn) An isolated microorganism comprising a *Propionibacteria* strain selected from the group consisting of strains P169, P170, P179, P195, P261, and genetic equivalents thereof.

2. (Withdrawn) An isolated microorganism of claim 1, wherein the strain comprises strain P169.

3. (Currently Amended) A method of feeding a first-ruminant, which comprises feeding the ruminant an effective amount of a microorganism comprising a propionibacteria strain selected from the group consisting of strains P169, P170, P179, P195, and P261 of the genus *Propionibacterium*, which has the following characteristics: (1) producing at least 0.9% (vol/vol) propionate in sodium lactate broth, and (2) producing at least 0.2% (vol/vol) propionate in rumen fluid (in vitro).

4. (Currently Amended) The method of claim 3, wherein the strain comprises strain P169 has a group I profile produced by *Xba* I digests of genomic DNA as shown in Figures 1-2 and Table 3.

5. (Currently Amended) The method of claim 3, wherein the first ruminant is a bovine.

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6. (Currently Amended) The method of claim 3, wherein the ~~first~~-ruminant is fed the microorganisms such that the amount of microorganism delivered to the ~~first~~-ruminant is about  $6 \times 10^9$  CFU to about  $6 \times 10^{12}$  CFU/animal/day.

7. (Currently Amended) The method of claim 6, wherein the ~~first~~-ruminant is fed the microorganisms such that the amount of microorganism delivered to the ~~first~~-ruminant is about  $6 \times 10^{11}$  CFU/animal/day.

8. (Currently Amended) The method of claim 3, wherein the ~~first~~-ruminant is fed 17 g of a 1:10 mixture of the microorganism, which has been freeze-dried and which is at a concentration of  $3.5 \times 10^{10}$  CFU/g, and a carrier on a daily basis.

9. (Currently Amended) The method of claim 3, wherein the ~~first~~-ruminant is fed the microorganism from -2 to 12 weeks postpartum.

10. (Currently Amended) A method of feeding a ~~first~~-ruminant, which comprises:  
testing the ruminant for at least one of energy balance, plasma non-esterified fatty acids levels, and plasma leptin level, then  
feeding to the ~~first~~-ruminant an effective amount of a microorganism of the genus *Propionibacterium*,

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after the feeding, testing the ~~first~~ ruminant for at least one of energy balance, plasma non-esterified fatty acids levels, and plasma leptin level, and

wherein the feeding of the microorganism increases obtaining a statistically significant improvement in at least one of energy balance, plasma non-esterified fatty acids levels, and plasma leptin level in the ruminant after it is fed the microorganism when compared to the ~~respective~~ energy balance, plasma non-esterified fatty acids levels, and plasma leptin level in the ~~a second~~ ruminant before it is ~~not~~ fed the microorganism.

11. (Currently Amended) The method of claim 10, wherein the energy balance is ~~increased~~ improved.

12. (Currently Amended) The method of claim 10, wherein at week 1 postpartum the plasma non-esterified fatty acids levels are increased.

13. (Previously Presented) The method of claim 10, wherein the plasma leptin level is increased.

14. (Currently Amended) The method of claim 10, wherein the microorganism fed ~~comprises is~~ *P. acidipropionici* or *P. jensenii*.

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15. (Currently Amended) The method of claim 14, wherein the microorganism fed comprises a ~~propionibacteria strain selected from the group consisting of strains P169, P170, P179, P195, and P261~~ of the genus *Propionibacterium*, which has the following characteristics:  
(1) producing at least 0.9% (vol/vol) propionate in sodium lactate broth, and (2) producing at least 0.2% (vol/vol) propionate in rumen fluid (in vitro).

16. (Currently Amended) The method of claim 15, wherein the ~~*P. acidipropionici* fed comprises strain P169~~ strain has a group I profile produced by *Xba* I digests of genomic DNA as shown in Figures 1-2 and Table 3.

17. (Currently Amended) The method of claim 10, wherein the ~~first and second ruminants are~~ is a bovine.

18. (Currently Amended) The method of claim 10, wherein the ~~first~~ ruminant is fed the microorganism at a level such that the ~~first~~ ruminant is dosed daily with about  $6 \times 10^9$  CFU to about  $6 \times 10^{12}$  CFU/animal/day.

19. (Currently Amended) The method of claim 18, wherein the ~~first~~ ruminant is fed the microorganism at a level such that the ruminant is dosed daily with about  $6 \times 10^{11}$  CFU/animal/day.

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20. (Currently Amended) The method of claim 18, wherein the ~~first~~-ruminant is fed the microorganism until populations of  $10^5$  to  $10^8$  CFU/ml ruminal fluid are established in the rumen of the ~~first animal~~ ruminant.

21. (Currently Amended) The method of claim 10, wherein the ~~first~~-ruminant is fed 17 g of a 1:10 mixture of the microorganism, which has been freeze-dried and which is at a concentration of about  $3.5 \times 10^{10}$  CFU/g, and a carrier on a daily basis.

22. (Currently Amended) The method of claim 10, wherein the ~~first~~-ruminant is fed the microorganism from -2 to 12 weeks postpartum.

23. (Currently Amended) A method of enhancing the protein content of milk produced by a ~~first~~-ruminant, the method comprising:

(a) milking the ruminant; then

(b) determining a percent of protein in a milk produced after the ruminant is fed the microorganism; then

(a c) feeding an effective amount of a microorganism of the genus *Propionibacterium* to the ~~first~~-ruminant; then

(b d) milking the ~~first~~-ruminant; and then

(e e) determining a percent of protein in a milk produced after the ~~first~~-ruminant is fed the microorganism, wherein during at least the first week of lactation, there is a statistically

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significant increase in the percent of protein in the milk produced by the first-ruminant after it is fed the microorganism is greater than the percent of protein in the milk produced by a second the ruminant before it is not fed the microorganism.

24. (Currently Amended) The method of claim 23, further comprising:  
before and after feeding the ruminant, testing a fat content of a milk produced by the ruminant,  
wherein the percent of fat in the milk produced by the first-ruminant after it is fed the  
microorganism is about 6% greater for at least the first 12 weeks of lactation than the percent of  
fat in the milk produced by the second ruminant before it is fed the microorganism.

25. (Currently Amended) The method of claim 23, before and after feeding the ruminant,  
testing the percent of solids-non-fat content of a milk produced by the ruminant, wherein during  
the first week of lactation, there is a statistically significant increase in the percent of solids-non-  
fat in the milk produced by the first-ruminant after it is fed the microorganism is substantially  
greater than the percent of solids-non-fat in milk produced by the second-ruminant before it is  
fed the microorganism.

26. (Currently Amended) The method of claim 23, wherein the first-ruminant is fed the  
microorganism at a level such that the first-ruminant is dosed daily with about  $6 \times 10^9$  CFU to  
about  $6 \times 10^{12}$  CFU/animal/day.

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27. (Currently Amended) The method of claim 26, wherein the ~~first~~ ruminant is fed the microorganism at a level such that the ~~first~~ ruminant is dosed daily with about  $6 \times 10^{11}$

CFU/animal/day.

28. (Currently Amended) The method of claim 23, wherein the ~~first~~ ruminant is fed the microorganism from -2 to 12 weeks postpartum.

29. (Currently Amended) The method of claim 23, wherein the ~~first and second~~ ruminants ~~are~~ is a bovine.

30. (Withdrawn) A method of feeding a ruminant, the method comprising:  
feeding an isolated microorganism of the genus *Propionibacterium* to the ruminant, consumption of the microorganism effective to support production of a first milk by the ruminant, the first milk having a first protein concentration, the first protein concentration greater than a second protein concentration in a second milk that is producible by the ruminant that has not been fed the microorganism.

31. (Withdrawn) A method of claim 30, wherein a percent of fat in the first milk produced by the ruminant fed the microorganism is substantially the same as in the second milk.

32. (Withdrawn) A method of claim 30, wherein the ruminant fed is a bovine.

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33. (Withdrawn) A feed composition, the feed composition comprising:
- (a) an isolated microorganism comprising a *Propionibacteria* strain selected from the group consisting of strains P169, P170, P179, P195, P261, and genetic equivalents thereof; and
  - (b) a carrier.
34. (Withdrawn) A feed composition of claim 33, wherein the *Propionibacteria* strain comprises strain P169.
35. (Withdrawn) A method of forming a direct fed, the method comprising:
- (a) growing, in a liquid nutrient broth, a culture including an isolated microorganism comprising a *Propionibacteria* strain selected from the group consisting of strains P169, P170, P179, P195, P261, and genetic equivalents thereof; and
  - (b) separating the microorganism from the liquid nutrient broth.
36. (Withdrawn) A method of claim 35, wherein the *Propionibacteria* strain comprises strain P169.
37. (Withdrawn) A method of claim 35, wherein the culture is grown to a level of about  $5 \times 10^8$  x CFU/ml to about  $4 \times 10^9$  CFU/ml.

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38. (Withdrawn) A method of claim 37, wherein the culture is grown to a level of  $2 \times 10^9$  CFU/ml.

39. (Withdrawn) A method of claim 35, further comprising freeze-drying the microorganisms to form the direct fed.

40. (Withdrawn) A method of claim 39, further comprising adding the freeze-dried microorganisms to a carrier.

41. (Currently Amended) The method of claim 3, further comprising:

before and after feeding the first-ruminant the microorganism, testing the first-ruminant  
for at least one of energy balance, plasma non-esterified fatty acids levels, and plasma leptin level;

wherein the feeding of the microorganism increases obtaining a statistically significant  
improvement in at least one of energy balance, plasma non-esterified fatty acids levels, and  
plasma leptin level in the first-ruminant <sup>in an amount effective to</sup> after it is fed the microorganism when compared to the  
respective energy balance, plasma non-esterified fatty acids levels, and plasma leptin level in a  
~~second the ruminant not before it is fed the microorganism.~~

42. (Currently Amended) The method of claim 41, wherein the energy balance is ~~increased~~  
improved.

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43. (Currently Amended) The method of claim 41, wherein at week 1 postpartum the plasma non-esterified fatty acids levels are increased.

44. (Previously Presented) The method of claim 41, wherein the plasma leptin level is increased.

45. (Currently Amended) The method of claim 3, wherein the ~~first~~-ruminant is fed the microorganism until populations of  $10^5$  to  $10^8$  CFU/ml ruminal fluid are established in the rumen of the ~~first~~-ruminant.

46. (Currently Amended) The method of claim 3, further comprising:  
before and after feeding the ~~first~~-ruminant, testing a protein content of a milk produced by the ~~first~~-ruminant,

wherein during at least the first week of lactation, there is a statistically significant increase in the percent of protein in the milk produced by the ~~first~~-ruminant after the feeding of the microorganism ~~has an enhanced protein content~~ when compared to a milk produced by the a ~~second~~-ruminant ~~not before it is~~ fed the microorganism.

47. (Currently Amended) The method of claim 3, further comprising:

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before and after feeding the first-ruminant, testing a fat content of a milk produced by the first-ruminant;

wherein ~~the~~ milk produced by the ~~first~~-ruminant after the feeding of the microorganism has ~~a higher~~ about 6% greater percent of fat for at least the first 12 weeks of lactation when compared to a milk produced by ~~a second the~~ ruminant ~~not before it is~~ fed the microorganism.

48. (Currently Amended) The method of claim 3, further comprising:

before and after feeding the first-ruminant, testing ~~a~~ the percent of solids-non-fat content of a milk produced by the first-ruminant;

wherein during the first week of lactation, the milk produced by the ~~first~~-ruminant after the feeding of the microorganism has a ~~substantially greater percent of~~ statistically significant increase in solids-non-fat when compared to a milk produced by ~~a second the~~ ruminant ~~not before it is~~ fed the microorganism.

49. (Currently Amended) A method of feeding a first-ruminant, comprising feeding the first ruminant an effective amount of a <sup>colony</sup> microorganism of the genus *Propionibacterium* comprising a ~~propionibacteria~~-strain having a group I profile produced by *Xba* I digests of genomic DNA as shown in Figures 1-2 and Table 3.

50. (Currently Amended) The method of claim 49, wherein the microorganism ~~comprises a~~ ~~propionibacteria strain selected from the group consisting of strains P169, P170, P179, P195, and~~

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P261 has the following characteristics: (1) producing at least 0.9% (vol/vol) propionate in sodium lactate broth, and (2) producing at least 0.2% (vol/vol) propionate in rumen fluid (in vitro).

51. (Currently Amended) The method of claim 50, wherein the strain ~~comprises strain P169~~ has at least one of the following characteristics: (1) is isolated from the rumen, (2) has the ability to survive and grow in the rumen, (3) <sup>can</sup> has the ability to be grown commercially, and (4) has the ability to survive a freeze-drying process.

52. (Currently Amended) The method of claim 49, wherein the ~~first~~-ruminant is a bovine.

53. (Currently Amended) The method of claim 49, wherein the ~~first~~-ruminant is fed the microorganism such that the amount of microorganism delivered to the ~~first~~-ruminant is about  $6 \times 10^9$  CFU to about  $6 \times 10^{12}$  CFU/animal/day.

54. (Currently Amended) The method of claim 53, wherein the ~~first~~-ruminant is fed the microorganism such that the amount of microorganism delivered to the ~~first~~-ruminant is about  $6 \times 10^{11}$  CFU/animal/day.

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55. (Currently Amended) The method of claim 49, wherein the ~~first~~-ruminant is fed 17 g of a 1:10 mixture of the microorganism, which has been freeze-dried and which is at a concentration of  $3.5 \times 10^{10}$  CFU/g, and a carrier on a daily basis.

56. (Currently Amended) The method of claim 49, wherein the ~~first~~-ruminant is fed the microorganism from -2 to 12 weeks postpartum.

57. (Currently Amended) The method of claim 49, further comprising:

before and after feeding the ~~first~~-ruminant the isolated microorganism, testing the ~~first~~ ruminant for at least one of energy balance, plasma non-esterified fatty acids levels, and plasma leptin level; and

~~wherein the feeding of the microorganism increases~~ obtaining a statistically significant increase in at least one of energy balance, plasma non-esterified fatty acids levels, and plasma leptin level in the ruminant after it is fed the microorganism when compared to the ~~respective~~ energy balance, plasma non-esterified fatty acids levels, and plasma leptin level in ~~a second~~ the ruminant ~~not before it is~~ fed the microorganism.

58. (Currently Amended) The method of claim 57, wherein the energy balance is ~~increased~~ improved.

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59. (Currently Amended) The method of claim 57, wherein at week 1 postpartum the plasma non-esterified fatty acids levels are increased.

60. (Previously Presented) The method of claim 57, wherein the plasma leptin level is increased.

61. (Currently Amended) The method of claim 49, wherein the ~~first~~-ruminant is fed the microorganism until populations of  $10^5$  to  $10^8$  CFU/ml ruminal fluid are established in the rumen of the ~~first~~-ruminant.

62. (Currently Amended) The method of claim 49, further comprising:  
before and after feeding the ruminant, testing a protein content of a milk produced by the ruminant, wherein a milk produced by the ~~first~~-ruminant after it is fed the microorganism has an ~~enhanced~~ a statistically significant increase in protein content when compared to a milk produced by a ~~second~~ the ruminant ~~not before it is~~ -fed the microorganism.

63. (Currently Amended) The method of claim 49, further comprising:  
before and after feeding the ruminant, testing a fat content of a milk produced by the ruminant, wherein a milk produced by the ~~first~~-ruminant after it is fed the microorganism has about 6% a higher percent of fat when compared to a milk produced by a ~~second~~ the ruminant ~~not before it is~~ fed the microorganism.

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64. (Currently Amended) The method of claim 49, further comprising:

before and after feeding the ruminant, testing the percent of solids-non-fat content of a  
milk produced by the ruminant, wherein during the first week of lactation, a milk produced by  
the ruminant after it is fed the microorganism has a substantially greater statistically significant  
increase in the percent of solids-non-fat when compared to a milk produced by a second the  
ruminant not before it is fed the microorganism.